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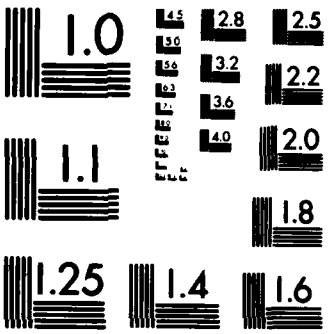

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

We have been examining the oxidation of ammonia to nitrite by oxygen radicals generated by the xanthine oxidase reaction. This oxidation ($NH_3 \rightarrow NO_2^-$), which can easily be demonstrated, is inhibited by superoxide dismutase, or by catalase, or by scavengers of the hydroxyl radical. We conclude that the iron-catalyzed reduction of H_2O_2 to OH^- + OH· by O_2^- is

20. Abstract (continued)

involved and that OH· is the first oxidant of NH_3. When NH_3 is replaced by NH_2OH we see NO_2^- production which is inhibited by superoxide dismutase but not by catalase. In this case we conclude that O_2^-, per se, can oxidize NH_2OH to NO_2^-. We have proposed a mechanism which includes the following intermediates:

$$H_2N\cdot,\ H_2NOH,\ HONOOH \text{ and finally } NO_2^-$$

This is of interest because O_2^-, H_2O_2 and OH· are known to be generated in cells and our mechanism provides a route which can explain the endogenous production of NO_2^-, which has previously been noted.

We have been reinvestigating the killing of E. coli by paraquat. Our earlier studies showed that the lethality of paraquat was dependent upon O_2 and an electron source and was decreased by elevated intracellular levels of superoxide dismutase. All of this, plus measurements of cyanide-resistant respiration, showed that O_2^- was essential for expression of the lethality of paraquat. This work was done in a nutrient broth medium. We now see that paraquat is much more lethal in the nutrient broth medium than it is in a simpler Vogel/Bonner medium. Indeed there is a heat stable and dialyzable factor in nutrient broth which appears essential for expression of paraquat lethality. This factor, once identified, should greatly increase our understanding of the mechanism of cell killing by O_2^-. We have begun to isolate this factor.

Originator furnished keywords include:

TABLE OF CONTENTS

TOXICITY, MUTAGENESIS AND AGING DUE TO ENDOGENOUS OXYGEN RADICALS

Final Report

Irwin Fridovich

December 14, 1984

U. S. ARMY RESEARCH OFFICE

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THIS VIEW, OPINIONS, AND/OR FINDINGS CONTAINED IN THIS REPORT ARE THOSE OF THE AUTHOR(S) AND SHOULD NOT BE CONSTRUED AS AN OFFICIAL DEPARTMENT OF THE ARMY POSITION, POLICY, OR DECISION, UNLESS SO DESIGNATED BY OTHER DOCUMENTATION.

C. List of All Publications during Tenure of Contract

Robertson, P., Jr., and I. Fridovich, A reaction of the superoxide radical with tetrapyrroles. Arch. Biochem. Biophys. 213, 353-357 (1982).

Archibald, F. S., and I. Fridovich, The scavenging of superoxide radical by manganous complexes: In vitro. Arch. Biochem. Biophys. 214, 452-462 (1982).

Archibald, F. S., and I. Fridovich, Investigations of the state of the manganese in Lactobacillus plantarum. Arch. Biochem. Biophys. 215, 589-596 (1982).

DiGuiseppi, J., and I. Fridovich, Oxygen toxicity in Streptococcus sanguis: the relative importance of superoxide and hydroxyl radicals. J. Biol. Chem. 257, 4046-4051 (1982).

Kono, Y., and Fridovich, Superoxide radical inhibits catalase. J. Biol. Chem. 257, 5751-5754 (1982).

Cudd, A. and I. Fridovich, Electrostatic interactions in the reaction mechanism of bovine erythrocyte superoxide dismutase. J. Biol. Chem. 257, 11443-11447 (1982).

Cudd, A., and Fridovich, I., Parallel electrostatic effects in the interactions of superoxide with cytochrome c and with superoxide dismutase. FEBS Lett. 144, 181-182 (1982).

Kirby, T. W., and I. Fridovich, A picomolar spectrophotometric assay for superoxide dismutase. Anal. Biochem. 127, 435-440 (1982).

Benovic, J., T. Tillman, A. Cudd and I. Fridovich, Electrostatic facilitation of the reaction catalyzed by the manganese-containing and the iron-containing superoxide dismutases. Arch. Biochem. Biophys. 221, 329-332 (1983).

Blum, J., and I. Fridovich, Superoxide, hydrogen peroxide and oxygen toxicity in two free-living nematode species. Arch. Biochem. Biophys. 222, 35-43 (1983).

Fridovich, I., Superoxide radical: an endogenous toxicant. Ann. Rev. Pharmacol. Toxicol. 23, 239-257 (1983).

Archibald, F. S., and I. Fridovich, Oxygen radicals, oxygen toxicity and the life of microorganisms. Acta Medica Portuguesa 4, 101-112 (1983).

Kono, Y., and I. Fridovich, Isolation and characterization of the pseudocatalase of Lactobacillus plantarum: a new manganese-containing enzyme. J. Biol. Chem. 258, 6015-6019 (1983).

Kono, Y., and I. Fridovich, Inhibition and reactivation of Mn-catalase. Implications for valence changes at the active site manganese. J. Biol. Chem. 258, 13646-13648 (1983).

Rabinowitch, H. D., D. A. Clare, J. D. Crapo and I. Fridovich, Positive correlation between superoxide dismutase and resistance to paraquat toxicity in the green alga Chlorella sorokiniana. Arch. Biochem. Biophys. 225, 640-648 (1983).

Kono, Y., and I. Fridovich, The functional significance of manganese catalase in Lactobacillus plantarum. J. Bacteriol. 155, 742-746 (1983).

Rabinowitch, H., and I. Fridovich, Superoxide radical, superoxide dismutases and oxygen toxicity in plants. Photochem. Photobiol. 37, 679-690 (1983).

DiGuiseppi, J., and I. Fridovich, The toxicology of molecular oxygen. CRC CRITICAL REVIEWS IN TOXICOLOGY 12, 315-342 (1984).

Clare, D. A., J. Blum and I. Fridovich, A hybrid superoxide dismutase containing both functional iron and manganese. J. Biol. Chem. 259, 5932-5936 (1984).

Picker, S. D., and I. Fridovich, On the mechanism of production of superoxide radical by reaction mixtures containing NADH, phenazine methosulfate, and nitroblue tetrazolium. Arch. Biochem. Biophys. 228, 155-158 (1984).

Blum, J., and I. Fridovich, Enzyme defenses against oxygen toxicity in the hydrothermal vent animals Riftia pachyptila and Calyptogena magnifica. Arch. Biochem. Biophys. 228, 617-620 (1984).

Clare, D. A., H. D. Rabinowitch and I. Fridovich, Superoxide dismutase and chilling injury in Chlorella ellipsoidea. Arch. Biochem. Biophys. 231, 158-163 (1984).

Darr, D. J., and I. Fridovich, Vanadate and molybdate stimulate the oxidation of NADH by superoxide radical. Arch. Biochem. Biophys. 232, 562-565 (1984).

Clare, D. A., M. N. Duong, D. Darr, F. Archibald and I. Fridovich, Effects of molecular oxygen on detection of superoxide radical with nitroblue tetrazolium and on activity stains for catalase. Anal. Biochem. 140, 532-537 (1984).

Pugh, S. Y. R., J. L. DiGuiseppi and I. Fridovich, Induction of superoxide dismutases in Escherichia coli by manganese and iron. J. Bacteriol., 160, 137-142 (1984).

Clare, D. A., F. A. Archibald and I. Fridovich, Effects of molecular oxygen on detection of superoxide radical with nitroblue tetrazolium and on activity stains for catalase. Anal. Biochem. 140, 532-537 (1984).

Blum, J., and I. Fridovich, Inactivation of glutathione peroxidase by superoxide radical. Arch. Biochem.Biophys., submitted (1984).

Brawn, M. K., and I. Fridovich, Increased superoxide radical production evokes inducible DNA repair in Escherichia coli. J. Biol. Chem., in press, 1984.

Nagano, T. and I. Fridovich, Does the aerobic xanthine oxidase reaction generate singlet oxygen. Photochem. Photobiol., in press, 1984.

Nagano, T., and I. Fridovich, Superoxide radical from xanthine oxidase acting upon lumazine. J. Free Rad. Biol. Med., in press, 1984.

Pugh, S. Y. R., and I. Fridovich, Induction of superoxide dismutase in Escherichia coli by metal chelators. J. Bacteriol., in press, 1984.

Rabinowitch, H. D., and I. Fridovich, Growth of Chlorella sorokiniana in the presence of sulfite elevates cell content of superoxide dismutase and imparts resistance towards paraquat. Planta, in press, 1984.

Darr, D., and Fridovich, I., Inhibition of catalase by 3,3'-diamino-benzidine. Biochem. J., in press, 1984.

Fridovich, I., Superoxide dismutases. Advan. Enzymol., in press (1984).

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Effects of Molecular Oxygen on Detection of Superoxide Radical with Nitroblue Tetrazolium and on Activity Stains for Catalase[1]

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The usual method of staining polyacrylamide gel electropherograms for superoxide dismutase activity utilizes a photochemical flux of O_2^- to reduce nitroblue tetrazolium. Superoxide dismutases intercept O_2^-, preventing formazan production and thus causing achromatic bands. In the presence of H_2O_2, catalases also yield achromatic bands during this staining procedure. This is due to local elevation of pO_2 by the catalatic decomposition of H_2O_2. O_2, in turn, inhibits the reduction of the tetrazolium by O_2^-. This phenomenon provides a new activity stain for catalase. A previously described activity stain for catalase has also been reexamined and significantly improved.

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Increased Superoxide Radical Production Evokes Inducible DNA Repair in *Escherichia coli**

(Received for publication, June 6, 1984)

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Paraquat induced the SOS response in *Escherichia coli*. This was measured in terms of acquired resistance towards UV lethality in a wild-type strain and in terms of appearance of β-galactosidase activity in a *din*::Mu *d*(Ap *lac*) fusion strain. However measured, the induction of the SOS response by paraquat was entirely dioxygen-dependent; whereas induction of the SOS response by mitomycin C was independent of the presence of dioxygen. As expected, *recA*(Def) and *lexA*(Ind⁻) isogenic strains did not show the SOS response. It appears likely that O_2^-, whose intracellular production is increased by paraquat, leads to DNA damage which in turn induces the SOS response.

Rabinowitch, H. D., and I. Fridovich, Growth of Chlorella sorokiniana in the presence of sulfite elevates cell content of superoxide dismutase and imparts resistance towards paraquat. Planta, in press, 1984.

Abstract

1. Growth of Chlorella sorokiniana in the presence of 7.5 mM sulfite, which halved the growth rate while doubling the superoxide dismutase EC #1.15.1.1 content per cell, rendered the cells resistant to the toxic effects of 30 μM paraquat.

2. While increasing total superoxide dismutase content, sulfite increased the relative amount of the H_2O_2-resistant manganese-containing superoxide dismutase.

3. It appears that O_2^- may be involved in mediating the toxicity of SO_2 in this green alga.

Key Words: Chlorella - Chlorophyta, paraquat, sulfite, superoxide dismutase

Darr, D., and Fridovich, I., Inhibition of catalase by 3,3'-diaminobenzidine. Biochem. J., in press, 1984.

The superoxide radical (O_2^-) is a frequently encountered intermediate of the reduction of dioxygen and it poses a threat to living cells, much as does H_2O_2. Metalloenzymes, called superoxide dismutases (SODs), provide a defense against O_2^- and are found in virtually all organisms. These enzymes, properly called superoxide/superoxide oxidoreductases, catalyze the conversion of O_2^- to $H_2O_2 + O_2$ and operate close to the diffusion limit. A decade has passed since the last review on SODs appeared in ADVANCES IN ENZYMOLOGY (1). Interest in these enzymes has grown steadily and rapidly. We will now survey some of the work fueled by this interest.

Diaminobenzidine (DAB) has repeatedly been used as a chromogenic substrate for peroxidases and for the peroxidatic activity of glutaraldehyde-treated catalase (Graham and Karnovsky, 1966; Fahimi, 1968; Novikoff and Goldfischer, 1969; Herzog and Fahimi, 1974). DAB plus horseradish peroxidase (HRP) has also been used to provide a negative stain for catalase activity on polyacrylamide gels (Gregory and Fridovich, 1974). In this method, gel electropherograms were soaked first in DAB plus HRP and then in H_2O_2. Zones containing catalase would become depleted of H_2O_2 and thus not show the chromogenic peroxidation of DAB by HRP, leaving achromatic zones against a uniformly stained background. It was subsequently noticed that changing the order of application of these reagents, such that DAB was the last reagent applied, markedly increased the sensitivity of this negative stain for catalase activity (Clare et al., 1984). This result was explained on the basis of the inhibition of catalase by DAB. Since the inhibition of catalase by DAB had not previously been reported, we undertook an investigation of this inhibition and also examined the effects of related compounds. The results of this study, which indicated that DAB inhibits catalase, both reversibly and irreversibly, and a mechanism consistent with these results, are presented below.

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